
CHROMATOGRAM

Retention time: 4 (-), 4.5 (+)

OTHER SUBSTANCES

Simultaneous: 4-hydroxypropranolol

KEY WORDS

derivatization; normal phase; chiral

REFERENCE

Wilson, M.J.; Walle, T. Silica gel high-performance liquid chromatography for the simultaneous determination of propranolol and 4-hydroxypropranolol enantiomers after chiral derivatization, *J. Chromatogr.*, **1984**, *310*, 424–430.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.1

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclimine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipranone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylethylgonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, naltrexone, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscaine, orphenadrine, oxeladin, oxprenolol, oxymetazoline, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutaramide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxylbenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaline, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, propiridine, propiomazine, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine,

thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 cellulose tris(3,5-difluorophenylcarbamate)

Mobile phase: Hexane:isopropanol 90:10

Flow rate: 0.5

Detector: UV

CHROMATOGRAM

Retention time: 16 (+), 32 (-)

KEY WORDS

chiral

REFERENCE

Okamoto, Y.; Aburatani, R.; Hatano, K.; Hatada, K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J. Liq. Chromatogr.*, **1988**, 11, 2147–2163.

SAMPLE

Matrix: solutions

Sample preparation: Evaporate an aliquot of a solution in MeCN containing 62.5 ng drug to dryness under a stream of nitrogen at room temperature, add 200 µL saturated sodium carbonate, add 200 µL 4% (-)-menthyl chloroformate in MeCN, vortex for 30 s, add an excess amount of 4-hydroxy-L-proline, vortex for 30 s, centrifuge for 3 min, inject a 10–25 µL aliquot of the upper layer.

HPLC VARIABLES

Guard column: 50 × 4.6 Pellicular ODS (Whatman)

Column: 100 × 4.6 5 µm Partisil 5 ODS3

Mobile phase: MeCN:water 60:40

Flow rate: 1

Injection volume: 10–25

Detector: F ex 200 nm no emission filter

CHROMATOGRAM

Retention time: 32 (-), 35 (+)

OTHER SUBSTANCES

Simultaneous: metoprolol, toliprolol

KEY WORDS

derivatization; chiral

REFERENCE

Mehvar, R. Stereospecific liquid chromatographic analysis of racemic adrenergic drugs utilizing precolumn derivatization with (-)-menthyl chloroformate, *J. Chromatogr.*, **1989**, 493, 402–408.

SAMPLE

Matrix: solutions

Sample preparation: Mix 20 μL of a solution of propranolol in buffer with 20 μL 4 mM (+)-1-(9-fluorenyl)ethyl chloroformate in dry acetone, let stand at room temperature for 10 min, inject a 10 μL aliquot. (Buffer was 100 mM boric acid/sodium bicarbonate buffer, adjusted to pH 8.5 with NaOH.)

HPLC VARIABLES

Column: 150 \times 4 MicroPak SP C8

Mobile phase: MeCN:20 mM pH 4.0 sodium acetate 70:30

Flow rate: 2

Injection volume: 10

Detector: F ex 265 em 345

CHROMATOGRAM

Retention time: 6.7, 7.2 (enantiomers)

Limit of detection: 1 pmole

KEY WORDS

derivatization; chiral

REFERENCE

Lai,F.; Mayer,A.; Sheehan,T. Chiral separation and detection enhancement of propranolol using automated pre-column derivatization, *J.Pharm.Biomed.Anal.*, **1993**, 11, 117-120.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 \times 3.2 7 μm SI 100 ODS (not commercially available)

Column: 150 \times 3.2 7 μm SI 100 ODS (not commercially available)

Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)

Flow rate: 0.5-1

Detector: UV 210, 225, 286

CHROMATOGRAM

Retention time: 2.0

Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, salicylamide, temazepam, tetracaine, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, 17, 4131-4144.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 \times 3.9 5 μm Nova-Pak C18

Mobile phase: MeOH:buffer 50:50 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 4 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)

Column temperature: 30

Flow rate: 1

Injection volume: 100

Detector: UV 220

CHROMATOGRAM**Retention time:** k' 2.84**OTHER SUBSTANCES****Also analyzed:** alprenolol, betaxolol, bopindolol, tertatolol**REFERENCE**Hamoir, T.; Verlinden, Y.; Massart, D. L. Reversed-phase liquid chromatography of β -adrenergic blocking drugs in the presence of a tailing suppressor, *J. Chromatogr. Sci.*, **1994**, 32, 14–20.**SAMPLE****Matrix:** solutions**HPLC VARIABLES****Column:** 250 \times 4.6 Zorbax RX**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.**Column temperature:** 30**Flow rate:** 2**Detector:** UV 210**OTHER SUBSTANCES****Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitrityline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyryl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephentyoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thiorida-

zine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tran-
lycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphen-
idyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine,
warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**,
18, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 10 µm Chiralpak AD (Daicel)

Mobile phase: Carbon dioxide:MeOH:diethylamine 69.5:30:0.5

Flow rate: 2

Detector: UV 210

CHROMATOGRAM

Retention time: 2.224, 2.596 (enantiomers)

KEY WORDS

SFC; chiral

REFERENCE

Kot,A.; Sandra,P.; Venema,A. Sub- and supercritical fluid chromatography on packed columns: A versatile tool
for the enantioselective separation of basic and acidic drugs, *J.Chromatogr.Sci.*, **1994**, 32, 439–448.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 25 µg/mL solution in MeCN:water 40:60, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 3 µm silica (Phenomenex)

Mobile phase: MeCN:6.25 mM pH 3.0 phosphate buffer 40:60

Flow rate: 1

Injection volume: 50

Detector: UV 254

CHROMATOGRAM

Retention time: 7.12

OTHER SUBSTANCES

Also analyzed: atenolol, clonidine, diltiazem, metoprolol, nifedipine, prazosin, verapamil

REFERENCE

Simmons,B.R.; Stewart,J.T. HPLC separation of selected cardiovascular agents on underivatized silica using
an aqueous organic mobile phase, *J.Liq.Chromatogr.*, **1994**, 17, 2675–2690.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 40 µL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 µm Hypersil MOS C-8

Mobile phase: MeOH:water 70:30 containing 0.02% dimethyloctylamine, 25 mM sodium hexa-
nesulfonate, and 20 mM acetic acid

Flow rate: 1

Injection volume: 40

Detector: UV 288

CHROMATOGRAM**Retention time:** 6.4

OTHER SUBSTANCES**Simultaneous:** alprenolol (F ex 275 em 305), atenolol (F ex 275 em 305), pindolol (F ex 275 em 305)

REFERENCE

Adson,A.; Burton,P.S.; Raub,T.J.; Barsuhn,C.L.; Audus,K.L.; Ho,N.F.H. Passive diffusion of weak organic electrolytes across Caco-2 cell monolayers: Uncoupling the contributions of hydrodynamic, transcellular, and paracellular barriers, *J.Pharm.Sci.*, **1995**, *84*, 1197–1204.

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 150 × 4.6 ChyRoSine-A 2 (Sedere)**Mobile phase:** Carbon dioxide:MeOH containing 1% n-propylamine 88:12**Flow rate:** 4**Detector:** UV 224

CHROMATOGRAM**Retention time:** k' 19.8

KEY WORDSSFC; pump head cooled at -5°; α = 2.07; chiral

REFERENCE

Bargmann-Leyder,N.; Sella,C.; Bauer,D.; Tambuté,A.; Caude,M. Supercritical fluid chromatographic separation of β -blockers on Chyrosine-A: Investigation of the chiral recognition mechanism using molecular modeling, *Anal.Chem.*, **1995**, *67*, 952–958.

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 62 × 2 packed with chiral packing (Prepare packing by dissolving 4-chloro-3-methyl-phenylcarbamate cellulose in THF, coat on Nucleosil 1000-7, dry at 60° for 3 h under reduced pressure.)**Mobile phase:** Hexane:isopropanol:diethylamine 90:10:0.1**Flow rate:** 0.1**Injection volume:** 20**Detector:** UV 254

CHROMATOGRAM**Retention time:** k' 3.30

KEY WORDSnarrow-bore; chiral; α 1.21

REFERENCE

Chankvetadze,B.; Chankvetadze,L.; Sidamonidze,S.; Yashima,E.; Okamoto,Y. Enantioseparation of some chiral pharmaceuticals using narrow-bore liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 695–699.

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 Chirex 3014 (Phenomenex)

Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 70:25:5 (EtOH/trifluoroacetic acid was premixed 20:1.)

Flow rate: 0.7-1

Injection volume: 20

Detector: UV 292

KEY WORDS

chiral; $\alpha = 1.11$ for enantiomers

REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, 18, 649-671.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Spherisorb S5SCX

Mobile phase: MeOH:MeCN:water 40:40:20 containing 25 mM perchloric acid

Flow rate: 2

Detector: F ex 215 no emission filter

CHROMATOGRAM

Retention time: 5.5

Internal standard: benzimidazole (7)

OTHER SUBSTANCES

Simultaneous: flecainide

REFERENCE

Croes,K.; McCarthy,P.T.; Flanagan,R.J. HPLC of basic drugs and quaternary ammonium compounds on micro-particulate strong cation-exchange materials using methanolic or aqueous methanol eluents containing an ionic modifier, *J.Chromatogr.A*, **1995**, 693, 289-306.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μ L aliquot of a 1 mg/mL solution.

HPLC VARIABLES

Column: 250 × 4.6 10 μ m Chiralcel OD

Mobile phase: Hexane:isopropanol:diethylamine 60:40:0.1

Flow rate: 0.5

Injection volume: 20

Detector: UV 290

CHROMATOGRAM

Retention time: k' 0.90, 1.52 (enantiomers)

KEY WORDS

chiral

REFERENCE

Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β -blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, 708, 253-261.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4 5 μ m CHIRAL-AGP (ChromTech)

Mobile phase: Isopropanol:96 mM pH 4.1 acetate buffer 0.5:99.5

Flow rate: 0.9

Injection volume: 20

Detector: UV 225

CHROMATOGRAM

Retention time: k' 7.04, 10.7 (enantiomers)

KEY WORDS

chiral

REFERENCE

Hermansson,J.; Grahn,A. Optimization of the separation of enantiomers of basic drugs. Retention mechanisms and dynamic modification of the chiral bonding properties on an α_1 -acid glycoprotein column, *J.Chromatogr.A*, **1995**, 694, 57-69.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 \times 4.6 12 μ m 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)

Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: k' 21.88

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripeleennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1 -acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, 9, 211-215.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Supelcosil LC-DP (A) or 250 \times 4.5 μ m LiChrospher 100 RP-8 (B)

Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

Flow rate: 0.6

Injection volume: 25

Detector: UV 229

CHROMATOGRAM

Retention time: 9.19 (A), 4.91 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphen-oxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flur-azepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloper- idol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxy- chloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazin- dol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimiprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, me- tronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymet- azoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, phen- iramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltolox- amine, phenytol, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, protriptyline, quazepam, quinidine, quinine, raceme- thorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertra- line, sotalol, spironolactone, sulfapyrazone, sulindac, temazepam, terbutaline, terfenadine, tet- racaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluorpromazine, tri- meprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 3.9 5 μm C8 Symmetry end-capped (prepared in the laboratory from Waters silica)

Mobile phase: MeOH:20 mM pH 7.00 potassium phosphate buffer 65:35

Column temperature: 23 ± 0.5

Flow rate: 1

Detector: UV

CHROMATOGRAM

Retention time: 1.5

OTHER SUBSTANCES

Simultaneous: amitriptyline

REFERENCE

O'Gara, J.E.; Alden, B.A.; Walter, T.H.; Petersen, J.S.; Niederländer, C.L.; Neue, U.D. Simple preparation of a C₈ HPLC stationary phase with an internal polar functional group, *Anal. Chem.*, **1995**, 67, 3809–3813.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Sumchiral CSP 10 (Sumika Chemical Analysis Service)

Mobile phase: n-Hexane:1,2-dichloroethane:EtOH:trifluoroacetic acid 250:140:10:1

Flow rate: 1

Detector: UV 230-280

CHROMATOGRAM

Retention time: k' 10.54

KEY WORDS

chiral; $\alpha = 1.13$

REFERENCE

Oi,N.; Kitahara,H.; Aoki,F. Direct enantiomer separations by high-performance liquid chromatography with chiral urea derivatives as stationary phases, *J.Chromatogr.A*, **1995**, 694, 129–134.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 cellulose 3,5-dimethylphenylcarbamate/10-undecenoate bonded to allylsilica

Mobile phase: Heptane:isopropanol:diethylamine 80:20:0.1

Flow rate: 1

Injection volume: 1000

Detector: UV 254

CHROMATOGRAM

Retention time: k' 1.91

KEY WORDS

chiral; $\alpha = 1.18$

REFERENCE

Oliveros,L.; Lopez,P.; Minguillon,C.; Franco,P. Chiral chromatographic discrimination ability of a cellulose 3,5-dimethylphenylcarbamate/10-undecenoate mixed derivative fixed on several chromatographic matrices, *J.Liq.Chromatogr.*, **1995**, 18, 1521–1532.

SAMPLE

Matrix: solutions

Sample preparation: Mix 10 μ L of a 1 mM amine solution in MeCN:water:triethylamine 50:50:2 with 10 μ L 5 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in MeCN, heat at 55° for 10 min, add 480 μ L 1 M acetic acid in MeCN:water 50:50, dilute 10-fold with MeCN, inject a 5 μ L aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole, (R)-(-)-NBD-PyNCS, is as follows. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (*J. Med. Chem.* 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-fluoro-7-nitro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0–10°, stir at room temperature for 30 min, remove the MeCN by evaporation

under reduced pressure, dissolve the residue in 50 mL water, extract 4 times with 80 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as dark red crystals (mp 178-181°) (Analyst 1992, 117, 727). Add 100 μ L thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole as red crystals (mp 165-170°).

HPLC VARIABLES

Column: 150 \times 4.6 5 μ m Inertsil ODS-80A

Mobile phase: MeCN:water:trifluoroacetic acid 40:60:0.05

Column temperature: 40

Flow rate: 1

Injection volume: 5

Detector: F ex 490 em 530

CHROMATOGRAM

Retention time: 10.5 ((R)-(+)-propranolol), 11.5 ((S)-(-)-propranolol)

OTHER SUBSTANCES

Simultaneous: alprenolol

KEY WORDS

derivatization; chiral

REFERENCE

Toyooka,T.; Liu,Y.-M. Development of optically active fluorescent Edman-type reagents, *Analyst*, **1995**, *120*, 385-390.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 1-10 μ g/mL solution in water, inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 5 μ m Hypersil SCX/C18

Mobile phase: MeCN:25 mM pH 3 Na₂HPO₄ 50:50

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.81

OTHER SUBSTANCES

Also analyzed: amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, imipramine, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

KEY WORDS

effect of mobile phase pH on capacity factor is discussed

REFERENCE

Walshe,M.; Kelly,M.T.; Smyth,M.R.; Ritchie,H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *708*, 31-40.

SAMPLE

Matrix: solutions

Sample preparation: Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmole acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmole sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

HPLC VARIABLES

Column: 250 \times 4.5 μm Zorbax ODS

Mobile phase: MeCN:water 70:30

Flow rate: 0.8

Injection volume: 100

Detector: UV 230, F ex 270 em 350

CHROMATOGRAM

Retention time: k' 8.7 (S-(-)), 10.7 (R-(+))

OTHER SUBSTANCES

Simultaneous: alprenolol

KEY WORDS

derivatization; chiral; F not much more sensitive than UV; $\alpha = 1.23$

REFERENCE

Büschges, R.; Linde, H.; Mutschler, E.; Spahn-Langguth, H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J. Chromatogr. A*, **1996**, 725, 323–334.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 20 μL aliquot of a 100–500 $\mu\text{g/mL}$ solution in mobile phase.

HPLC VARIABLES

Column: 100 \times 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)

Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60

Flow rate: 0.5–2

Injection volume: 20

Detector: UV 254

CHROMATOGRAM

Retention time: k' 2.40

OTHER SUBSTANCES

Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil

Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, 70, 2092–2099.

SAMPLE

Matrix: tissue

Sample preparation: Weigh out brain tissue and homogenize in 4 volumes 400 mM perchloric acid using a Tamson motor-driven PTFE/glass homogenizer at 1400 rpm to give a final tissue concentration of 25 mg/mL in the perchloric acid. For each 1 mL of homogenate add 40 μ L 31.4 μ g/mL clenbuterol in 200 mM sulfuric acid, centrifuge at 3000 g for 15 min. Remove 1 mL supernatant and add it to 10 μ L 10 M NaOH and 350 μ L buffer, vortex for 10 s, add 8 mL diethyl ether, shake mechanically for 45 min, centrifuge at 2000 g for 8 min. Remove the organic layer and add it to 200 μ L 200 mM sulfuric acid, shake mechanically for 15 min, centrifuge at 2000 g for 8 min. Remove the aqueous layer and heat it at 45° for 1 h to remove traces of ether, inject a 50 μ L aliquot. (Buffer was 90 g sodium carbonate and 32 g potassium carbonate in 1 L, pH 9.0.)

HPLC VARIABLES

Guard column: 20 \times 4.6 5 μ m LC-18-DB (Supelchem)

Column: 250 \times 4.6 5 μ m LC-18-DB (Supelchem)

Mobile phase: MeCN:50 mM NaH₂PO₄:triethylamine 35:65:0.1, adjusted to pH 3.0 with orthophosphoric acid

Column temperature: 40

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 7.3

Internal standard: clenbuterol (4.8)

Limit of detection: 22 ng/mL

OTHER SUBSTANCES

Simultaneous: labetalol

KEY WORDS

rat; brain

REFERENCE

Botterblom,M.H.A.; Feenstra,M.G.P.; Erdsieck-Ernste,E.B.H.W. Determination of propranolol, labetalol and clenbuterol in rat brain by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, 613, 121–126.

SAMPLE

Matrix: urine

Sample preparation: 1 mL Urine + 10 mg β -glucuronidase/arylsulfatase (Helix pomatia, Sigma), heat at 37° overnight, add an equal volume of buffer, centrifuge at 2000 g for 5 min, inject an aliquot of the supernatant onto column A with mobile phase A and elute to waste.

After 2.5 min backflush the contents of column A onto column B with mobile phase B, monitor the effluent from column B. Re-equilibrate both columns for 12.5 min before the next injection. (Buffer was 200 mM boric acid adjusted to pH 9.5 with 5 M NaOH.)

HPLC VARIABLES

Column: A 10 × 4.6 5 μm Spherisorb cyanopropyl; B 250 × 4.6 Capcell Pak C18 UG-120 (Shiseido)

Mobile phase: A water; B Gradient. MeCN:buffer from 3:97 to 30:70 over 30 min, to 40:60 over 8 min (Buffer was 3.4 mL/L phosphoric acid adjusted to pH 3.0 with 5 M NaOH.)

Flow rate: A 1.25; B 1

Injection volume: 100

Detector: UV 220

CHROMATOGRAM

Retention time: 15.3

Limit of detection: 250 ng/mL

OTHER SUBSTANCES

Extracted: acebutolol, alprenolol, amphetamine, atenolol, bopindolol, codeine, ephedrine, labetalol, metoprolol, morphine, nadolol, oxprenolol, pindolol, timolol

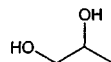
KEY WORDS

column-switching

REFERENCE

Saareninen, M.T.; Sirén, H.; Riekkola, M.-L. Screening and determination of β-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, *J. Chromatogr. B*, **1995**, 664, 341–346.

Propylene glycol



Molecular formula: C₃H₈O₂

Molecular weight: 76.10

CAS Registry No.: 57-55-6

Merck Index: 8040

SAMPLE

Matrix: formulations

Sample preparation: Remove the water from 10 μL syrup under reduced pressure for 10 min, reconstitute with 2 mL pyridine. Remove a 25 μL aliquot and add it to 75 μL reagent, shake well, let stand at room temperature for 10 min, evaporate to dryness under reduced pressure at room temperature, flush the tube with a stream of air or nitrogen, add 2 mL 5% sodium carbonate solution containing 2.5 mg/mL 4-dimethylaminopyridine, shake or sonicate for 5 min, extract with 2 mL chloroform. Wash the extract with 2 mL 5% sodium bicarbonate solution, wash twice with 3 mL portions of 50 mM HCl containing 5% NaCl, inject an aliquot. (Prepare reagent by dissolving 100 mg 4-nitrobenzoyl chloride in pyridine with gentle warming.)

HPLC VARIABLES

Column: 150 × 3 5 μm LiChrosorb SI 60

Mobile phase: n-Hexane:chloroform:MeCN 10:3:1.9 containing 0.1% water

Flow rate: 1.4

Injection volume: 50

Detector: UV 260

CHROMATOGRAM

Retention time: 1.5

OTHER SUBSTANCES

Simultaneous: dextrose, fructose, glycerin, saccharose, sorbitol

KEY WORDS

syrup; derivatization; normal phase

REFERENCE

Nachtmann,F.; Budna,K.W. Sensitive determination of derivatized carbohydrates by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, 136, 279–287.

SAMPLE

Matrix: formulations

Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 2 mL MeOH and 20 mL water. Dilute formulation ten-fold with water, add a 0.5 mL aliquot to the SPE cartridge, elute with three 1 mL portions of water, inject a 10 μ L aliquot of the eluate.

HPLC VARIABLES

Column: 8 mm i.d. C18 radial compression (Waters)

Mobile phase: Water

Flow rate: 1

Injection volume: 10

Detector: RI

CHROMATOGRAM

Retention time: 4.75

OTHER SUBSTANCES

Simultaneous: dihydroxyacetone, dioxane, ethylene glycol, formic acid, glyceraldehyde, glycerol, methylglyoxal

KEY WORDS

SPE

REFERENCE

Bobin,M.F.; Martini,M.C.; Gudefin,A.; Cotte,J. Dosage de la dihydroxyacétone dans les émulsions [Assay of dihydroxyacetone in emulsions], *Farmaco.[Prat.]*, **1983**, 38, 403–414.

SAMPLE

Matrix: solutions

Sample preparation: Add 0.5 mmole propylene glycol to a solution of 0.5 g 3,5-dinitrobenzoyl chloride in 30 mL pyridine, mix well, heat at 60° for 15 min, adjust pH to 2.5 with 2 M HCl (Caution! Exothermic reaction!), cool to room temperature, add 25 mL butyl acetate, shake for 3 min. Remove the organic layer and add it to 50 mL 1% sodium carbonate solution, shake for 2 min. Remove the organic layer and add it to 25 mL 0.25 M sulfuric acid, shake for 2 min. Remove the organic layer and add it to 10 mL water, shake for 1 min, inject a 10 μ L aliquot of the organic layer.

HPLC VARIABLES

Column: 2000 \times 2.1 Corasil II

Mobile phase: Heptane:ethyl acetate 75:25

Flow rate: 1.7

Injection volume: 10

Detector: UV 254

CHROMATOGRAM

Retention time: 5

OTHER SUBSTANCES

Simultaneous: diethylene glycol, ethylene glycol

KEY WORDS

derivatization; normal phase

REFERENCE

Carey, M.A.; Persinger, H.E. Liquid chromatographic determination of traces of aliphatic carbonyl compounds and glycols as derivatives that contain the dinitrophenyl group, *J. Chromatogr. Sci.*, **1972**, *10*, 537-543.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in water containing 5 mg/mL galactose, inject a 20 μ L aliquot.

HPLC VARIABLES

Column: 250 \times 4 Aminex carbohydrate HPX-87 (BioRad)

Mobile phase: water

Column temperature: 80

Flow rate: 0.2

Injection volume: 20

Detector: RI

CHROMATOGRAM

Retention time: 8.5

Internal standard: galactose (7)

OTHER SUBSTANCES

Simultaneous: arabinose, arabitol, dextran, dextrose, erythritol, fructose, fucose, galactan, galactitol, galactomannan, gentibiose, iditol, lyxose, maltose, maltotriose, mannose, melezitose, melibiose, pentaerythritol, pullulan, raffinose, rhamnose, ribitol, sorbitol, sorbose, sucrose, trehalose, turanose, xylitol, xylose

Noninterfering: xylan

Interfering: glycol, mannitol

KEY WORDS

detector temp 30°

REFERENCE

Dokladalova, J.; Barton, A.Y.; Mackenzie, E.A. High pressure liquid chromatographic determination of sorbitol in bulk sorbitol, *J. Assoc. Off. Anal. Chem.*, **1980**, *63*, 664-666.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 \times 7.8 Sarasep CAR-Ca (MetaChem)

Mobile phase: 50 μ g/mL dicalcium EDTA

Column temperature: 80

Flow rate: 0.7

Detector: RI

CHROMATOGRAM

Retention time: 14.9

OTHER SUBSTANCES

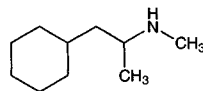
Simultaneous: acetone, acetonitrile, adonitol, arabinose, arabitol, cellobiose, dextrose, erythritol, ethanol, ethylene glycol, fructose, fucose, galactinol, galactitol, galactopinitol, galactose, gentianose, gentiobiose, glucoheptose, glycerol, isomaltose, ketose, lactitol, lactose, lactulose, maltitol, maltose, maltotetraose, maltotriose, mannoheptulose, mannose, melezitose, melibiose, methanol, myo-inositol, nystose, palatinol, palatinose, perseitol, pinitol, raffinose, rhamnose, ribose, sedoheptulosan, sedoheptulose, sorbitol, sorbose, styachyose, sucrose, tagatose, trehalose, turanose, volemitol, xylitol, xylose

Interfering: mannitol

REFERENCE

MetaChem Catalog, 1994, p. 65.

Propylhexedrine



Molecular formula: $C_{10}H_{21}N$

Molecular weight: 155.28

CAS Registry No.: 3595-11-7, 101-40-6

Merck Index: 8045

Lednicer No.: 1 37

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 1.5 mg compound in 1 mL reagent, add 3 μ L triethylamine, sonicate for 20 min, add 3 μ L diethylamine, let stand for 15 min, inject an aliquot. (Reagent was 2 mg/mL (R)-(-)-(naphth-1-yl)ethylisocyanate solution in dry chloroform:DMF 80:20.)

HPLC VARIABLES

Column: 200 \times 4.6 Silica 100 RP 18

Mobile phase: MeOH:water 70:30

Flow rate: 1.5

Detector: UV 254

CHROMATOGRAM

Retention time: k' 9.26, k' 10.19 (enantiomers)

OTHER SUBSTANCES

Also analyzed: atenolol, methylphenidate, metipranolol, pindolol, propranolol, talinolol

KEY WORDS

derivatization; chiral

REFERENCE

Jira,T.; Toll,C.; Vogt,C.; Beyrich,T. Zur Trennung einiger racemischer β -Blocker und α -Sympathikomimetika durch HPLC nach Derivatisierung [The separation of some racemic β -blockers and α -sympathomimetics with HPLC following derivatization], *Pharmazie*, 1991, 46, 432-434.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 1.5 mg compound in 1 mL reagent, add 3 μ L triethylamine, sonicate for 20 min, add 3 μ L diethylamine, let stand for 15 min, inject an aliquot. (Reagent was 2 mg/mL (R)-(+)-1-phenylethylisocyanate ((R)-(+)- α -methylbenzylisocyanate) solution in dry chloroform:DMF 80:20.)

HPLC VARIABLES

Column: 200 \times 4.6 Silica 100 RP 18

Mobile phase: MeOH:water 70:30

Flow rate: 1

Detector: UV 254

CHROMATOGRAM

Retention time: k' 4.36, k' 4.73 (enantiomers)

OTHER SUBSTANCES

Simultaneous: propranolol

KEY WORDS

derivatization; chiral

REFERENCE

Jira,T.; Toll,C.; Vogt,C.; Beyrich,T. Zur Trennung einiger racemischer β -Blocker und α -Sympathikomimetika durch HPLC nach Derivatisierung [The separation of some racemic β -blockers and α -sympathomimetics with HPLC following derivatization], *Pharmazie*, **1991**, *46*, 432-434.

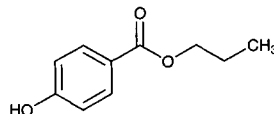
Propylparaben

Molecular formula: $C_{10}H_{12}O_3$

Molecular weight: 180.20

CAS Registry No.: 94-13-3

Merck Index: 8051

**SAMPLE**

Matrix: solutions

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

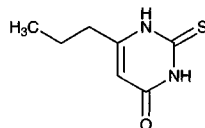
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbomal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenproporex, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendi-

metrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, pseudoephedrine, puromycin, pyrilamine, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233-242.

Propylthiouracil



Molecular formula: C₇H₁₀N₂OS

Molecular weight: 170.24

CAS Registry No.: 51-52-5

Merck Index: 8054

Lednicer No.: 1 265

SAMPLE

Matrix: blood

Sample preparation: 1 mL Plasma + 100 µL 1 M NaOH, shake, add 4 mL chloroform, vortex, centrifuge at 300 g for 10 min, discard the chloroform layer, add 300 µL 10% HCl to the aqueous layer, vortex, add 2 mL chloroform, vortex, centrifuge at 300 g for 10 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 30 µL MeOH, add 20 µL mobile phase, mix, inject a 20 µL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Newguard RP-18

Column: 250 × 4.6 Spheri-5 RP-18

Mobile phase: MeOH:50 mM pH 7.4 phosphate buffer 30:70

Column temperature: 37

Flow rate: 1.5

Injection volume: 20

Detector: UV 214

CHROMATOGRAM

Retention time: 7.25

Limit of detection: 40 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

Noninterfering: acetaminophen, albuterol, atenolol, bupivacaine, caffeine, carbamazepine, clonazepam, cortisone, diazepam, estriol, hydrocortisone, hyoscine, methimazole, metoprolol, phenobarbital, prednisolone, prednisone, progesterone, propranolol, ritodrine, verapamil

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Cannell,G.R.; Williams,J.P.; Yap,A.S.; Mortimer,R.H. Selective liquid chromatographic assay for propylthiouracil in plasma, *J.Chromatogr.*, **1991**, *564*, 310-314.

SAMPLE**Matrix:** blood**Sample preparation:** 500 μ L Plasma + 100 mg disodium EDTA + 3 mL ethyl acetate, vortex for 3 min, centrifuge at 5000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μ L MeOH, inject a 10 μ L aliquot.

HPLC VARIABLES**Guard column:** 25 \times 3 LiChroprep RP-18 (Merck)**Column:** 250 \times 4 10 μ m LiChrosorb RP-18**Mobile phase:** Gradient. MeOH:25 mM pH 3 phosphate buffer from 10:90 to 70:30 over 30 min**Flow rate:** 1**Injection volume:** 10**Detector:** UV 276

CHROMATOGRAM**Retention time:** 17.30**Limit of detection:** 200 ng/mL

OTHER SUBSTANCES**Extracted:** methimazole (UV 258), methylthiouracil, phenylthiouracil, thiouracil

KEY WORDS

cow; plasma

REFERENCE

Moretti,G.; Betto,P.; Cammarata,P.; Fracassi,F.; Giambenedetti,M.; Borghese,A. Determination of thyreostatic residues in cattle plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1993**, 616, 291–296.

SAMPLE**Matrix:** blood**Sample preparation:** 450 μ L Plasma + 60 μ L 100 mM HCl + 50 μ L 25 μ g/mL methylthiouracil in water, vortex for 10 s, add 6 mL dichloromethane:acetone 75:25, vortex for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 μ L water, vortex for 20 s, inject a 20 μ L aliquot.

HPLC VARIABLES**Guard column:** 5 μ m Lichrospher RP-18**Column:** 125 \times 4 5 μ m Lichrospher RP-18**Mobile phase:** THF:buffer 0.9:99.1, pH 6.0 \pm 0.1 (Buffer was 34 g KH₂PO₄ and 170 mL 200 mM NaOH, make up to 5 L with water.)**Flow rate:** 1.5**Injection volume:** 20**Detector:** UV 275

CHROMATOGRAM**Retention time:** 15.20**Internal standard:** methylthiouracil (2.60)**Limit of detection:** 5 ng/mL**Limit of quantitation:** 40 ng/mL

KEY WORDS

dog; plasma

REFERENCE

Kabanda,L.; De Muyck,C.; Lefebvre,R.A.; Remon,J.P. Validation of a HPLC method for the determination of propylthiouracil in plasma, *J.Liq.Chromatogr.*, **1994**, 17, 2069–2083.

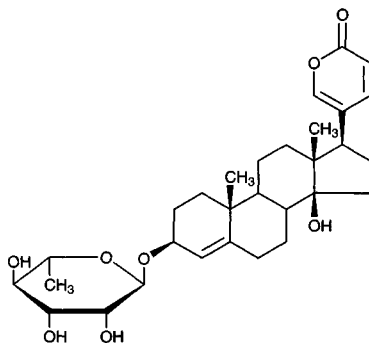
Proscillaridin

Molecular formula: $C_{30}H_{42}O_8$

Molecular weight: 530.66

CAS Registry No.: 466-06-8

Merck Index: 8060



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 \times 4.6 5 μ m Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 15.585

KEY WORDS

whole blood

REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149-163.

Protamine sulfate

CAS Registry No.: 9009-65-8

SAMPLE

Matrix: formulations

Sample preparation: Centrifuge 10 mL of an insulin formulation at 5° at 2000 rpm for 20 min, discard the supernatant, dissolve the pellet in 500 μ L 100 mM HCl, inject an aliquot.

HPLC VARIABLES**Column:** 250 × 4.6 5 µm Zorbax C8**Mobile phase:** MeCN:buffer 2.5:97.5 (Buffer was 100 mM NaH₂PO₄ adjusted to pH 2 with phosphoric acid.)**Flow rate:** 1**Detector:** UV 220

CHROMATOGRAM**Retention time:** 17.5, 21.5, 27, 42.5

KEY WORDSsalmon; fish

REFERENCE

Hoffmann,J.A.; Chance,R.E.; Johnson,M.G. Purification and analysis of the major components of chum salmon protamine contained in insulin formulations using high-performance liquid chromatography, *Protein Expr.Purif.*, **1990**, *1*, 127-133.

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 4.6 SynChropak RP C18 (Baxter)**Mobile phase:** Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.05% trifluoroacetic acid in isopropanol. A:B from 95:5 to 0:100 over 30 min.**Flow rate:** 1**Detector:** UV 210

CHROMATOGRAM**Retention time:** 6.5

OTHER SUBSTANCES**Simultaneous:** angiotensin, aprotinin, insulin B

REFERENCE

Baxter Scientific Products Catalog, 1992-3, p. 187.

SAMPLE**Matrix:** solutions

HPLC VARIABLES**Column:** 250 × 10 Vydac C18**Mobile phase:** Gradient. A was MeOH:water 10:90 containing 0.1% heptafluorobutyric acid. B was MeOH containing 0.1% heptafluorobutyric acid. A:B from 100:0 to 10:90 over 60 min, maintain at 10:90 for 5 min.**Detector:** UV 230

CHROMATOGRAM**Retention time:** 57

KEY WORDSfish; yellow perch; testes; semi-preparative

REFERENCE

Chao,H.; Davies,P.L. Amino acid sequence of the unique protamine from yellow perch, *FEBS Lett.*, **1992**, *299*, 166-168.

SAMPLE**Matrix:** solutions

Sample preparation: Centrifuge at 14000 rpm for 10 min, filter (0.22 μm), dilute with 10 mM HCl (if necessary), inject an aliquot.

HPLC VARIABLES

Column: 250 \times 4.6 Zorbax C8

Mobile phase: MeCN:100 mM pH 2 sodium phosphate buffer 2:98

Flow rate: 1

Detector: UV 215

CHROMATOGRAM

Retention time: 6, 8, 10, 18

OTHER SUBSTANCES

Simultaneous: insulinotropin

KEY WORDS

salmon

REFERENCE

Kim, Y.; Rose, C.A. Precipitation of insulinotropin in the presence of protamine: Effect of phenol and zinc on the isophane ratio and the insulinotropin concentration in the supernatant, *Pharm.Res.*, **1995**, *12*, 1284–1288.

Prothipendyl

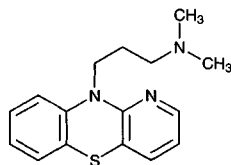
Molecular formula: $\text{C}_{16}\text{H}_{19}\text{N}_3\text{S}$

Molecular weight: 285.41

CAS Registry No.: 303-69-5, 1225-65-6 (HCl)

Merck Index: 8073

Lednicer No.: 1 430



SAMPLE

Matrix: solutions

Sample preparation: Prepare a 10 $\mu\text{g/mL}$ solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 \times 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 4.95

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipamone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine,

droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, mecllophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylegonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleppamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191-225.

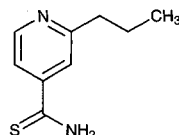
Protionamide

Molecular formula: $C_9H_{12}N_2S$

Molecular weight: 180.27

CAS Registry No.: 14222-60-7

Merck Index: 8076



SAMPLE

Matrix: blood

Sample preparation: Add 60 μ L 30% trichloroacetic acid to 300 μ L serum. Immediately vortex and centrifuge at 4° at 23000 g for 10 min. Mix 200 μ L supernatant with 45 μ L 1 M sodium bicarbonate and centrifuge. Inject a 50 μ L aliquot.

HPLC VARIABLES

Guard column: 5 \times 3.5 μ m Kromasil 100 C4

Column: 100 \times 3.5 μ m Kromasil 100 C4

Mobile phase: MeCN:buffer 25:75 (Buffer was 9.534 g sodium tetraborate and 6.4625 g dibutylamine in 1 liter water, adjusted to pH 8 with concentrated HCl.)

Flow rate: 0.6

Injection volume: 50

Detector: UV 291

CHROMATOGRAM

Retention time: 4.7

Limit of quantitation: 27 ng/mL

OTHER SUBSTANCES

Noninterfering: cefazimine, ofloxacin, rifabutin, thiacezone

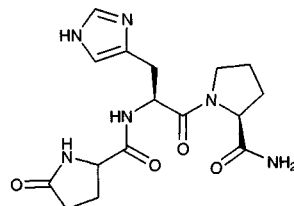
KEY WORDS

serum

REFERENCE

Bartels,H.; Bartels,R. Simple, rapid and sensitive determination of protonamide in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, 707, 338–341.

Protirelin

Molecular formula: $C_{16}H_{22}N_6O_4$ **Molecular weight:** 362.39**CAS Registry No.:** 24305-27-9**Merck Index:** 9720**SAMPLE****Matrix:** formulations

Sample preparation: Dilute 4 mL injection to 200 mL with water. Remove a 5 mL aliquot and add it to 10 mL 1 mg/mL p-aminobenzoic acid in water, make up to 200 mL with water, inject a 10 μ L aliquot.

HPLC VARIABLES**Column:** 150 \times 4.6 5 μ m Nucleosil C18

Mobile phase: MeCN:MeOH:buffer:triethylamine 4:4:92:0.01 (Buffer was 0.05% sodium octanesulfonate adjusted to pH 2.2 with 3 M phosphoric acid.)

Flow rate: 1.5**Injection volume:** 10**Detector:** UV 215**CHROMATOGRAM****Retention time:** 16**Internal standard:** p-aminobenzoic acid (9.5)**OTHER SUBSTANCES**

Simultaneous: benzaldehyde, benzoic acid, benzyl alcohol, degradation products.

KEY WORDS

stability-indicating; injections

REFERENCE

Rao,G.N.; Sutherland,J.W.; Menon,G.N. High-performance liquid chromatographic assay for thyrotropin releasing hormone and benzyl alcohol in injectable formulation, *Pharm.Res.*, **1987**, 4, 38–41.

SAMPLE**Matrix:** solutions**HPLC VARIABLES****Column:** 125 \times 4 mm Superspher 100 RP-18**Mobile phase:** MeCN:0.1% pH 2.0 trifluoroacetic acid 5:95**Flow rate:** 1**Detector:** UV 215**CHROMATOGRAM****Retention time:** 2.4**Limit of detection:** 500 nM

REFERENCE

Werner,U.; Kisel,T.; Stüber,W. Effects of peptide structure on transport properties of seven thyrotropin releasing hormone (TRH) analogues in a human intestinal cell line (Caco-2), *Pharm.Res.*, **1997**, *14*, 246–250.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: Gradient. A was 0.1% phosphoric acid. B was MeCN:0.1% phosphoric acid 70:30. A:B from 95:5 to 30:70 over 20 min.

Flow rate: 1

Detector: UV 206 or RIA

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES

Simultaneous: gonadorelin, somatostatin, substance P

REFERENCE

McDermott,J.R.; Smith,A.I.; Biggins,J.A.; Al-Noaemi,M.C.; Edwardson,J.A. Characterization and determination of neuropeptides by high-performance liquid chromatography and radioimmunoassay, *J.Chromatogr.*, **1981**, *222*, 371–379.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 TSKgel ODS-120T

Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.

Flow rate: 1

Detector: UV 220

CHROMATOGRAM

Retention time: 4

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin II, α-endorphin, β-endorphin, calcitonin (human), gonadorelin (LH-RH), somatostatin

REFERENCE

Varian Catalog, **1993**, p. 182.

Protriptyline

Molecular formula: C₁₉H₂₁N

Molecular weight: 263.38

CAS Registry No.: 438-60-8, 1225-55-4 (HCl)

Merck Index: 8088

Lednicer No.: 1 152

SAMPLE

Matrix: blood

